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Timing and sequence of postharvest fungicide and biocontrol agent applications for control of pear decay

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Abstract

Postharvest decay of pear fruit often originates at small wounds that occur during harvest and handling. Experiments were conducted to characterize the effect of timing of application of postharvest decay control materials, and to evaluate sequential postharvest applications of fungicides or fungicides and biocontrol agents. Fungicides and biocontrol agents were increasingly less effective when the period between harvest and application was prolonged. Thiabendazole (TBZ) applied to fruit without artificial wounding or inoculation effectively reduced decay when applied within 3 weeks or 6 weeks in 2 years of study. TBZ, fludioxonil, and pyrimethanil were effective in controlling decay at artificial wounds inoculated with *Penicillium expansum* up to 14 d after inoculation. Application of TBZ at harvest followed 3 weeks later by application of fludioxonil was superior to application of TBZ at harvest alone. Three yeast and one bacterial biocontrol agents reduced decay at pear wounds inoculated with *P. expansum* up to 14 d after inoculation with *P. expansum*, but were ineffective when applied at 28 d after inoculation. Of possible sequential arrangements of fungicide and biocontrol treatments, application of the most effective material promptly after harvest generally resulted in the highest level of decay control.

Keywords: Postharvest management; *Pyrus communis*; *Rodotorula glutinis*; *Cryptococcus infirmo-miniatum*; *Cryptococcus laurentii*; *Pseudomonas syringae*; Bio-Save 110

1. Introduction

Decay of pear fruit during long-term storage, caused by any of several fungal pathogens, can result in significant economic losses for pear producers (Kupferman, 1998). In order to reduce incidence of postharvest decay, fungicides or biocontrol agents may be applied to the fruit after harvest (Chand-Goyal and Spotts, 1997; Eckert and Sommer, 1967; Roberts, 1994). For most of the past three decades, most pears packed for long-term storage in the United States were treated with a benzimidazole fungicide (benomyl or thiabendazole [TBZ]). Recently, pyrimethanil and fludioxonil have been registered for postharvest application to pears in the United States and elsewhere, and have been shown to be effective against pear decay (Errampalli, 2003; Vostermans et al., 2005).

Harvest of pears takes place during a relatively narrow range of fruit maturity, followed by prompt cooling to remove field heat (Hansen and Mellenthin, 1979). Among the varying methods of postharvest handling employed by commercial operators, opportunities for application of decay control treatments typically occur (1) before fruit are placed in long-term storage, either as high-volume recirculating "drenches" while the fruit are in field bins or as in-line spray treatments during pre-storage sorting and sizing and (2) as in-line spray treatments immediately before fruit are packed into the boxes in which they are marketed. Many commercial pear operations store pears for extended periods in field bins because the large volume of pears harvested in the maturity period may require several months for sorting and packing to be completed, and because of uncertainty regarding the type of packaging that will be needed to fill specific market demands at the time of sale (E.A. Kupferman, personal communication). For various reasons, pears are often stored in field bins without postharvest fungicide treatment, receiving decay control treatment only as an in-line spray before final packing. These reasons include avoiding drench applications to minimize risk of accumulating spores of pathogens, especially of *Penicillium expansum*, washed from the surface of the fruit (Fidler et al., 1973), and avoiding pre-storage sizing to minimize risk of bruis-

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ing and surface injury during handling. Increased incidence of decay caused by *P. expansum* and by *Mucor piriformis* have been associated with pre-storage drenching in field bins (Sanderson et al., 1998; Xiao et al., 2004).

Since decay is often initiated at small wounds that occur during harvest and handling (Spotts et al., 1998), the timing of applications of postharvest decay control materials should be critical to successful decay control. The objectives of this study were to: (1) characterize the effects of delay in application of fungicides and biocontrol agents after harvest or after pathogen inoculation on pear postharvest decay and (2) to compare possible sequential arrangements of postharvest applications of two fungicides or a fungicide and a biocontrol agent for decay control.

2. Materials and methods

2.1. Timing of postharvest fungicide applications

2.1.1. TBZ timing with natural inoculum

At normal harvest maturity (71–62 N firmness) in 1996 and 1997, approximately 500 pears were harvested from each of five randomly arrayed mature 'Bosc' pear trees in an orchard at the Southern Oregon Research and Extension Center near Medford, and brought to the laboratory. One hundred fruit from each replicate tree were immediately treated with TBZ, applied as Mertect 340F (Syngenta Crop Protection, Greensboro, NC) at 1.25 mL L^{-1} (0.6 g active ingredient per litre applied) by spraying the fruit while traveling across a series of rotating brushes, simulating the common packinghouse treatment method. Fruit were then stored in polyethylene-lined fiberboard boxes in air at 0 °C. The remaining pears were stored at 0 °C. One hundred fruit from each replicate tree were removed from storage after 3, 6, and 9 weeks and treated with TBZ as described above. After 5 months of storage, all fruit were evaluated for incidence of decay lesions, and types of decay were identified.

2.1.2. TBZ, fludioxonil and pyrimethanil timing with artificial inoculation

In 1999, 2003 and 2004, 'Bosc' pears were harvested as described above and stored at 0 °C for 1 week. All fruit were then removed from storage and surface-disinfested by immersion in a 0.5% sodium hypochlorite solution for 2 min, then rinsed in fresh water. Each fruit was then wounded in three locations with a sterile finishing nail (2 mm diameter × 3 mm depth) and dipped in a spore suspension (1×10^7 conidia L^{-1}) of *P. expansum*. Conidia of *P. expansum* were obtained by washing the surfaces of 2-week-old colonies growing on potato dextrose agar at 20 °C, and adjusting conidial concentration in water with the aid of a hemacytometer. In 2004 the strain of *P. expansum* used was resistant to TBZ. From each of the five replicate trees, 25 fruit were wounded and treated with TBZ as described above, or with fludioxonil, applied as Scholar 50W (Syngenta Crop Protection, Greensboro, NC) at 0.6 g L^{-1} (0.3 g active ingredient per litre applied), or pyrimethanil, applied as Penbotec 400SC (Janssen Pharmaceutica, Titusville, NJ) at 2.5 mL L^{-1} (1.0 g active ingredient per litre applied). Treatments were applied to the fruit either shortly after inoculation (day 0) or at 1, 2, 7, 14 and 21 d after inoculation. All equipment was thoroughly cleaned with water between fungicide treatments. After treatment, fruit were stored in polyethylene-lined fiberboard boxes in air at 0 °C, and incidence of decay lesions at wounds was evaluated 2 months after inoculation.

2.2. Sequence of postharvest fungicide applications

The efficacy of various sequences of two postharvest treatments was compared by applying either water (control), TBZ (Mertect 340F) at 1.25 mL L^{-1}, or fludioxonil (Scholar 50W) at 0.6 g L^{-1} to 'Bosc' pears immediately after harvest followed by a second treatment with a different material at 3 weeks after harvest. In 1999 and 2000, treatments were applied to non-wounded fruit without artificial inoculation; in 2001 and 2003, each fruit was wounded immediately after harvest in three locations with a finishing nail (2 mm diameter × 3 mm depth) and dipped in a spore suspension of *P. expansum* (1×10^7 conidia L^{-1}) prior to initial treatment. All treatments were applied by spraying the fruit while traveling across a series of rotating brushes. Fruit were stored as described previously at 0 °C, and incidence of decay was evaluated after 2 months (wound-inoculated fruit) or 5 months (non-wounded fruit).

2.3. Timing of postharvest biocontrol agent applications

The yeasts *Rodotorula glutinis* and *Cryptococcus infirmo-miniatum* were obtained from R.A. Spotts, Oregon State University (Chand-Goyal and Spotts, 1997), and *Cryptococcus laurentii* was obtained from R.G. Roberts, USDA-ARS, Wenatchee WA (Roberts, 1990). The yeasts were grown on yeast malt dextrose agar (Difco) for 2 d at 20 °C, then suspended in water at concentrations adjusted to approximately 1–3 × 10^{11} cfu L^{-1} using a spectrophotometer (Sugar and Spotts, 1999). The formulated biocontrol product Bio-Save 110 (formerly Bio-Save 11) was obtained from EcoScience Corp., Worcester MA (subsequently produced by Village Farms LP, Longwood FL). This product contains the bacterium *Pseudomonas syringae* strain ESC-11 at a concentration of 9×10^{13} cfu kg^{-1} (Janisiewicz and Marchi, 1992; Jeffers and Hankinson, 1995), and was applied at 1.65 g L^{-1}.

'Bosc' pear fruit from five replicate orchard trees were surface-disinfested by immersion in a 0.5% sodium hypochlorite solution for 2 min, then rinsed in fresh water. Each fruit was then wounded in five locations with a sterile finishing nail (6 mm diameter × 3 mm depth) and inoculated by delivering 40 μL of a spore suspension of *P. expansum* (1×10^6 conidia L^{-1}) into each wound by micropipette. The conidial suspension of *P. expansum* was prepared as described above.

Immediately after inoculation with *P. expansum*, or 1, 7, 14, or 28 d after inoculation, suspensions of biocontrol agents (40 μL), at the concentrations described above, were added to the inoculated wounds. Ten fruit from each replicate (a total of 50 wounds) were used for each biocontrol agent at each application timing. Between pathogen inoculation and biocontrol treatment, and following biocontrol treatment, the fruit were stored in

Table 1
Effect of timing of postharvest application of thiabendazole (as Mertect 340F at 1.25 mL L^{-1}) on incidence of decay in 'Bosc' pear fruit after 5 months storage at 0 °C

Weeks between harvest and thiabendazole treatment	Decay incidence (% of fruit)[a]	
	1996	1997
Untreated	6.3 a	24.9 a
0	1.2 c	1.7 c
3	2.8 bc	4.8 bc
6	5.4 a	9.7 bc
9	5.1 ab	17.7 ab

[a] Blue mold (*P. expansum*) was the predominant type of decay in all treatments. Fruit were not artificially inoculated. Values within columns followed by the same letter are not significantly different according to Fisher's protected LSD test ($P > 0.05$).

polyethylene-lined fiberboard boxes in air at 0 °C. Decay lesions were measured using calipers after 2 months of storage.

2.4. Sequence of postharvest fungicide-biocontrol agent applications

The efficacy of various postharvest TBZ and Bio-Save 110 treatment sequences was compared by applying either water (control), TBZ (Mertect 340F, 1.25 mL L^{-1}), or Bio-Save 110 (1.65 g L^{-1}) to 'Bosc' pears immediately after harvest or 6 weeks after harvest (Table 5). For each treatment sequence, approximately 100 fruit from each of five replicate orchard trees were treated by passing the fruit through a spray while traveling across a series of rotating brushes. Fruit were neither artificially wounded nor artificially inoculated. Between and following treatments, all fruit were stored in fiberboard boxes with polyethylene liners in air at 0 °C. Decay incidence was evaluated after 5 months of storage.

3. Results and discussion

3.1. Timing of postharvest fungicide applications

3.1.1. TBZ timing with natural inoculum

In all experiments with natural inoculum, the predominant postharvest disease present was blue mold (*P. expansum*). Other types of decay, including gray mold (*Botrytis cinerea*), Cladosporium rot (*Cladosporium herbarum*), and decay of unknown causes, occurred at a very low incidence. TBZ treatment significantly reduced incidence of postharvest decay in experiments without artificial wounding or inoculation when applied 0 or 3 weeks after harvest in 1996, and up to 6 weeks after harvest in 1997, as compared to the untreated control (Table 1). While the incidence of decay was substantially higher in 1997 than in 1996, in both years there was a trend towards increased decay incidence when the period between harvest and TBZ application was prolonged.

3.1.2. TBZ, fludioxonil and pyrimethanil timing with artificial inoculation

In pears artificially wounded and inoculated with *P. expansum*, TBZ significantly reduced blue mold decay incidence through 14 d after pathogen inoculation in the 2 years in which a TBZ-sensitive strain was used (Table 2). TBZ was completely ineffective in reducing incidence of blue mold when a TBZ-resistant strain was used in 2004. In 2003, TBZ effectiveness diminished when treatments were applied at 7 or 14 d after inoculation. In contrast, fludioxonil and pyrimethanil completely suppressed infection when applied 7 d after pathogen inoculation in all three years of trial. At 14 d after inoculation, a small amount of decay appeared following fludioxonil treatment in one of three years; pyrimethanil at 14 d after inoculation suppressed only half of the decay in 2003, while providing complete suppression of the TBZ-resistant strain in 2004. In the one year in which the trial included fungicide treatment at 21 d after pathogen inoculation, the efficacy of both fludioxonil and pyrimethanil appeared to diminish (Table 2). Results of trials with and without artificial inoculation support the value of prompt application of postharvest fungicide treatments for decay control.

Table 2
Effect of timing of postharvest application of fungicides on incidence of blue mold decay in 'Bosc' pear fruit after 2 months storage at 0 °C

Days between inoculation and treatment	Treatment[a]	Percentage of wounds infected[b]		
		1999	2003	2004
0	Water	89.6 a	100.0 a	100.0 a
	Thiabendazole	0.8 b	0.0 b	100.0 a
	Fludioxonil	0.0 b	0.0 b	0.0 b
	Pyrimethanil	–	0.0 b	0.0 b
1	Water	96.0 a	100.0 a	100.0 a
	Thiabendazole	0.8 b	0.0 b	100.0 a
	Fludioxonil	0.0 b	0.0 b	0.0 b
	Pyrimethanil	–	0.0 b	0.0 b
2	Water	99.2 a	100.0 a	100.0 a
	Thiabendazole	0.0 b	0.0 b	100.0 a
	Fludioxonil	0.0 b	0.0 b	0.0 b
	Pyrimethanil	–	0.0 b	0.0 b
7	Water	94.4 a	100.0 a	100.0 a
	Thiabendazole	0.8 b	31.7 b	100.0 a
	Fludioxonil	0.0 b	0.0 c	0.0 b
	Pyrimethanil	–	0.0 c	0.0 b
14	Water	96.0 a	100.0 a	100.0 a
	Thiabendazole	0.0 b	68.3 b	100.0 a
	Fludioxonil	8.8 b	0.0 c	0.0 b
	Pyrimethanil	–	53.3 b	0.0 b
21	Water	–	–	100.0 a
	Thiabendazole	–	–	100.0 a
	Fludioxonil	–	–	86.7 ab
	Pyrimethanil	–	–	66.7 b

[a] Thiabendazole was applied as Mertect 340F at 1.25 mL L^{-1}; fludioxonil as Scholar 50W at 0.6 g L^{-1}; pyrimethanil as Penbotec 400SC at 2.5 mL L^{-1}.

[b] After harvest, each fruit was wounded with a nail (2 mm diameter × 3 mm depth) and dipped in a spore suspension (1×10^6 conidia L^{-1}) of *P. expansum* prior to fungicide treatment. In 2004 the strain of *P. expansum* used was resistant to thiabendazole. Decay was evaluated 2 months after pathogen inoculation. Fruit were stored in air at 0 °C. Values within columns followed by the same letter are not significantly different according to Fisher's protected LSD test ($P > 0.05$).

Table 3
Effect of postharvest fungicide treatment sequences on incidence of decay in 'Bosc' pears

Treatment and timing[a]		Percentage of fruit with decay lesions[b]			
At harvest	3 weeks after harvest	1999	2000	2001	2003
Water	Water	8.8 a	4.4 a	85.2 a	99.3 a
Water	Thiabendazole	4.1 b	3.1 a	–	94.7 b
Water	Fludioxonil	4.8 b	3.6 a	–	82.7 c
Thiabendazole	Water	1.2 c	0.8 b	29.4 b	40.7 d
Thiabendazole	Fludioxonil	1.4 c	1.0 b	11.0 c	13.3 e
Fludioxonil	Water	1.1 c	0.7 b	5.2 c	4.7 f
Fludioxonil	Thiabendazole	0.8 c	0.6 b	2.4 c	1.3 f

[a] In 1999 and 2000, treatments were applied to unwounded fruit without artificial inoculation. In 2001 and 2003, fruit were wounded with a nail (2 mm diameter × 3 mm depth) and dipped in a spore suspension of *P. expansum* (1×10^7 conidia L^{-1}) prior to treatment. Thiabendazole was applied as Mertect 340F at 1.25 mL L^{-1}, and fludioxonil as Scholar 50W at 0.6 g L^{-1} to fruit on rotating brushes passing through a treatment spray.

[b] Between and following treatments, all fruit were stored in fiberboard boxes with perforated polyethylene liners in air at 0 °C. Decay incidence was evaluated after 2 months of storage (wound-inoculated fruit) or 5 months of storage (non-wounded fruit). Values within columns followed by the same letter are not significantly different according to Fisher's protected LSD test ($P > 0.05$).

3.2. Sequence of postharvest fungicide applications

In one of 2 years of trials without artificial inoculation (1999), treatment with TBZ or fludioxonil applied at 3 weeks after harvest, without prior treatment at harvest, significantly reduced decay incidence (Table 3). However, in 2000 this treatment was not significantly different from the control. In the trial using inoculated fruit in 2003, although decay was significantly reduced by treatments at 3 weeks after harvest alone, decay incidence was still very high. Application of either TBZ or fludioxonil at harvest in trials without artificial inoculation (1999 and 2000) resulted in significant decay reduction, regardless of whether or not a fungicide treatment was applied at 3 weeks after harvest. In trials with artificial inoculation (2001 and 2003), the greatest decay control was observed where fludioxonil was applied at harvest. In 2001, fludioxonil treatment 3 weeks after harvest following TBZ treatment at harvest was not significantly different than treatments with fludioxonil at harvest, while in 2003 treatment with fludioxonil at harvest followed by TBZ 3 weeks later was more effective in reducing decay than treatment with TBZ at harvest followed by fludioxonil 3 weeks later (Table 3). These results support the overall greater value of fungicide applications immediately after harvest than 3 weeks after harvest.

3.3. Timing of postharvest biocontrol agent applications

All of the biocontrol agents tested provided significant decay reduction when applied up to 14 d after pathogen inoculation (Table 4). At 28 d after pathogen inoculation, all biocontrol treatments were ineffective. Since the most common mode of action for biocontrol agents in decay suppression appears to be competition for nutrients and space (Droby and Chalutz, 1994; Filonow, 1998; Mari et al., 2003), it is logical that the efficacy of biocontrol agents would depend upon their establishment in the wound site before the pathogen infection process has advanced into the host tissue beyond the point of being influenced by such competition.

3.4. Sequence of postharvest fungicide-biocontrol agent applications

In both years of study, treatments with either Bio-Save 110 or TBZ applied 6 weeks after harvest, with only water treatment at harvest, did not control decay (Table 5). Treatments with Bio-Save 110 at harvest followed by TBZ or water applied 6 weeks after harvest did not significantly improve decay control over the control treatment in 1996, but TBZ treatment at harvest followed by Bio-Save 110 at 6 weeks after harvest did result in

Table 4
Effect of timing of application of biocontrol agents on lesion development in 'Bosc' pears inoculated with *Penicillium expansum*

Days between pathogen inoculation and introduction of biocontrol agent	Average lesion diameter (mm)[a]			
	Rodotorula glutinis	*Cryptococcus infirmo-miniatum*	*Cryptococcus laurentii*	Bio-Save 110
0	0.0 a	0.7 a	0.0 a	7.5 a
1	4.2 b	7.3 b	3.0 ab	7.9 a
7	8.7 c	7.8 b	6.9 b	9.8 b
14	9.3 c	7.8 b	5.1 b	7.8 a
28	15.0 d	12.2 c	11.9 c	13.2 b
No biocontrol	14.4 d	14.4 c	14.4 c	14.4 b

[a] After harvest, each fruit was wounded with a nail (6 mm diameter × 3 mm depth) and inoculated with spore suspensions of *P. expansum* (1×10^7 conidia L^{-1}) by pipette. *R. glutinis*, *C. infirmo-miniatum*, or *C. laurentii* ($1–3 \times 10^{11}$ cfu L^{-1}), or Bio-Save 110 (1.65 g L^{-1} of product containing the bacterium *Pseudomonas syringae* strain ESC-11 at 9×10^{13} cfu kg^{-1}) were added to the same wounds at the indicated timings, and decay lesions were measured 2 months after pathogen inoculation. Fruit were stored in air at 0 °C. Values within columns followed by the same letter are not significantly different according to Fisher's Protected LSD test ($P > 0.05$).

Table 5
Effect of postharvest fungicide and biocontrol agent treatment sequences on incidence of decay in 'Bosc' pears

Treatment and timing[a]		Decay incidence (% of fruit)	
At harvest	6 weeks after harvest	1996	1997
Water	Water	7.8 a	10.0 a
Water	Bio-Save 110	8.1 a	12.4 a
Water	Thiabendazole	8.2 a	10.9 a
Bio-Save 110	Thiabendazole	6.7 a	4.8 b
Bio-Save 110	Water	6.7 a	4.1 b
Bio-Save 110	Bio-Save 110	–	1.5 b
Thiabendazole	Bio-Save 110	1.7 b	3.4 b
Thiabendazole	Water	4.5 ab	2.2 b
Thiabendazole	Thiabendazole	–	1.6 b

[a] Fruit were neither artificially wounded nor artificially inoculated. Between and following treatments, all fruit were stored in fiberboard boxes with perforated polyethylene liners in air at 0 °C. Thiabendazole was applied as Mertect 340F at 1.25 mL L^{-1}, and Bio-Save 110 at 1.65 g L^{-1} to fruit on rotating brushes passing through a spray application of the treatment solution. Bio-Save 110 contains the bacterium *Pseudomonas syringae* strain ESC-11 at 9×10^{13} cfu kg^{-1}. Decay incidence was evaluated after 5 months of storage. Blue mold (*P. expansum*) was the predominant type of decay which resulted in all treatments. Values within columns followed by the same letter are not significantly different according to Fisher's protected LSD test ($P > 0.05$).

significant decay reduction as compared to the control. In 1997, either Bio-Save 110 or TBZ treatment at harvest resulted in significant decay reduction, while subsequent treatment at 6 weeks after harvest did not further reduce decay. Treatments of TBZ or Bio-Save 110 applied 6 weeks after harvest in this experiment appeared to be of little value, while the same treatments applied at harvest, with the exception of Bio-Save 110 in 1996, significantly reduced decay.

4. Conclusions

It appears from these data that application of fungicides or biocontrol agents as soon as possible after harvest is likely to provide the most benefit in decay control; delay in application of treatments will exacerbate the risk of decay. While there is little evidence of benefit in protecting infections initiated at wounds occurring at harvest from treatments applied 6 weeks or more after harvest, decay control treatments applied after prolonged storage may protect new wounds occurring during final packing and transport. These data further suggest that when sequential applications of different decay control materials are made, the greatest benefit may be obtained from applying the most effective material as the earlier treatment.

Resistance to postharvest fungicides used on pome fruit has been reported from several locations in the United States and elsewhere (Bertrand and Saulie-Carter, 1978; Rosenberger and Meyer, 1981; Spotts and Cervantes, 1986; Viñas et al., 1991; Wicks, 1977). Resistance management includes the deployment of treatment materials with diverse modes of action (Fungicide Resistance Action Committee, 1998). Sequential applications of decay control materials with distinct modes of action may contribute to suppression of resistance development in pathogen populations.

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